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Lake origin determines *Daphnia* population growth under winter conditions

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In large oligotrophic lakes, growth rates of zooplankton populations decline to low or negative values in winter as a result of low food concentration and water temperature. Daphnia, a key species in the aquatic food web, has two strategies to overwinter: either by sexual reproduction resulting in diapause or as asexual clones in the open water. We investigated how asexually overwintering Daphnia clones, originating from different taxa and lakes with different trophic state, survive under winter conditions. We performed a laboratory experiment exposing D. hyalina and D. hyalina×galeata clones to low water temperature (5°C) and either no or low food (<0.1 mg C L^{-1}) conditions. We used clones from three pre-alpine lakes in Switzerland with contrasting trophic state: ultra-oligotrophic Lake Brienz, mesotrophic Lake Constance and eutrophic Greifensee. Our results show that Daphnia clones can withstand starvation for an average of 6 weeks under low food concentration and for almost 2 weeks under complete absence of food. Besides strong clonal variation, we found a significant effect of food concentration and its interaction with lake origin on population growth rate. Our study indicates that adaptation to local winter conditions is a key factor in defining the clonal, but not necessarily the taxonomic composition of a Daphnia population in unproductive lakes.

INTRODUCTION

The cladoceran *Daphnia*, a key species in the aquatic food web, has two overwintering strategies (De Meester *et al.*, 2006). One strategy involves sexually produced diapausing eggs that hatch when conditions ameliorate, for example, in the following spring, and another involves asexual females that survive the winter in open water. Although permanent large lakes favour asexual reproduction (Mort and Wolf, 1986), often both modes of reproduction are found together (Jankowski and Straile, 2004; Keller and Spaak, 2004). Sexual reproduction forms new genotypes through recombination, but is energetically costly (Lynch *et al.*, 1986) and requires the temporal and spatial co-occurrence of males and sexual females (Keller *et al.*, 2007). Overwintering asexual clones have to cope with strong selection factors: low water temperature and, at least in unproductive systems, low food concentration. In oligotrophic lakes, food concentration can drop below food threshold levels (Lampert, 1977, 1978; Gliwicz, 1990) for several consecutive months (Rellstab and Spaak, 2007). If overwintering asexual clones survive this period of starvation, they could have a competitive advantage over sexually produced clones in spring when conditions ameliorate, because they are already present in the water column and do not depend on hatching cues. Variation in the ability of clones and taxa to survive periods of starvation

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under low water temperature conditions can thus affect the clonal and taxonomic composition of *Daphnia* populations and influence the dynamics of the whole food web.

Several studies have been made of the effect of starvation on *Daphnia* (Elendt, 1989; Gliwicz, 1991; Epp, 1996), and many of them focus on short-time starvation (Plath, 1998) and subsequent refeeding (Bradley *et al.*, 1991). Resistance of daphnids to starvation depends on the amount of energy reserves, i.e. lipids (Lemcke and Lampert, 1975) and the allocation of this energy into reproduction or survival (Tessier *et al.*, 1983; Bradley *et al.*, 1991; McCauley and Nisbet, 1991). In general, smaller species and individuals show lower survival under starvation than larger ones (Gliwicz, 1991), and juveniles show lower survival than adults (Threlkeld, 1976). This is consistent with the size-efficiency hypothesis which states that larger bodied species or individuals should be superior, as the feeding rate increases faster than the respiration rate with increasing body size (Brooks and Dodson, 1965). Maternal effects (Mousseau and Fox, 1998) play an important role in the ability to survive starvation, at least in early life stages (Lynch and Ennis, 1983), because lipids are passed from the mother to her offspring (Tessier *et al.*, 1983). Starvation resistance can differ among *Daphnia* species (Threlkeld, 1976), but also intraspecific clonal variation has been shown (Epp, 1996).

Although periods of low food levels often occur in winter in pre-alpine lakes, none of the above-mentioned studies used water temperatures typical for that season. Temperature is an important factor influencing life-histories of *Daphnia* because it changes, for example, the processes of energy intake, assimilation and respiration losses (Burns, 1968; Yurista, 1999). Studies testing temperatures experienced in natural environments during winter are rare (Bottrell, 1975; Vijverberg, 1980; Goss and Bunting, 1983). Rising temperatures generally speed up rates of development or growth by decreasing parameters such as instar duration (Vijverberg, 1980), number of instars to first reproduction (Goss and Bunting, 1983) or egg development time (Bottrell, 1975), and increasing the clutch size (Goss and Bunting, 1983) and the growth rate (Vijverberg, 1980). Often these factors show an optimal response temperature which can be between 15 and 25°C depending on the *Daphnia* species. To our knowledge, no study exists that investigates the effect of temperature experienced by *Daphnia* during winter on its lifespan under constant food concentration, but it is generally assumed that the low temperature increases lifespan as a consequence of a lower "rate of living" (Pearl, 1928), as discussed in Lynch and Ennis, and Gliwicz *et al.* (Lynch and Ennis, 1983; Gliwicz *et al.*, 2001).

The pre-alpine lakes of Switzerland differ greatly in their trophic state and therefore provide an ideal opportunity to study the effect of low food conditions, or the complete absence of food, on the performance of different *Daphnia* taxa. Large lakes with low phosphorus content are dominated by *D. hyalina*, whereas *D. galeata* prefers more productive habitats. *Daphnia hyalina* × *galeata* hybrids are as common in Swiss lakes as in other European lakes (Schwenk and Spaak, 1995) and most frequent in lakes with the largest changes in magnitude of phosphorus content since the peak of eutrophication in the 1970s (Keller *et al.*, 2008). *Daphnia hyalina* has the tendency to overwinter asexually, whereas *D. galeata* relies more on resting eggs (Jankowski and Straile, 2004).

In the present study, we wanted to investigate how various clones from the *D. hyalina/galeata* species complex cope with low food concentration and the low water temperatures typical for winter in pre-alpine oligotrophic lakes. In particular, we were interested in the survival and reproduction of *Daphnia* under these circumstances. We hypothesized that (i) high mortality and no reproduction would be found at extremely low food conditions; (ii) *D. hyalina* clones would be more resistant to starvation than *D. hyalina* × *galeata* hybrids and (iii) clones originating from more oligotrophic lakes would be more resistant than clones from more productive systems. To test these hypotheses, we performed a starvation experiment using *D. hyalina* and *D. hyalina* × *galeata* clones from three lakes with contrasting productivity. To put our results into perspective, we present field data from the most unproductive system used in this study, ultra-oligotrophic Lake Brienz.

METHOD

Studied lakes

The *Daphnia* population of ultra-oligotrophic Lake Brienz (BRZ), $P_{tot} = 3.0\ \mu g\ L^{-1}$, $SRP = 0.9\ \mu g\ L^{-1}$, situated just north of the Swiss Alps, is dominated by *D. hyalina*, but also hybrids of *D. hyalina* × *galeata* and their backcrosses can be found (C. Rellstab, unpublished results). In recent years, during the phase of sexual reproduction in autumn, sexual individuals (males, and females with ephippia) represented, on average, 4% of the population (C. Rellstab, unpublished results). However, it is assumed that the main strategy to overwinter is as asexual females, as the lake does not provide ideal conditions for the production and hatching of diapausing eggs. Sixty percent of the recent ephippia found in the sediment do not contain eggs, most likely as a result of the low population density

(Rellstab *et al.*, 2007) reducing the chances of mating. Moreover, the high sedimentation rate and the great depth of the lake prevent hatching of the diapausing eggs in most locations (Rellstab *et al.*, 2007).

Mesotrophic Lake Constance (CON), $P_{tot} = 13\ \mu g\ L^{-1}$ (Stich, 2004), is situated at the border of Switzerland, Germany and Austria. All taxa of the *D. hyalina/galeata* species complex are present. *Daphnia galeata* is the predominant taxon in summer and produces dormant eggs thereafter. The native species *D. hyalina* is the most abundant in autumn and mainly overwinters asexually. Hybrids also occur throughout the year. Sexual stages in summer or autumn represent an average of 6–8% of the population (Jankowski and Straile, 2004).

Eutrophic Greifensee (GRE), $P_{tot} = 63\ \mu g\ L^{-1}$ (Keller *et al.*, 2008), is situated in the Swiss lowland. It is dominated by *D. hyalina* × *galeata* hybrids, but parental *D. galeata* and *D. hyalina* can also be found, with the latter being rare (Spaak *et al.*, 2001). Food conditions are sufficient for egg production throughout the winter. During the phase of sexual reproduction, the proportion of sexual stages reaches up to 6% of the population. This can happen up to three times a year, in spring, summer and autumn (P. Spaak, unpublished results).

Field data of Lake Brienz

Qualitative sampling of the *Daphnia* population in Lake Brienz was performed at least monthly from August 2003 to January 2006 using a single net with a mesh size of 250 μm. Up to 10 net tows from 70-0 m depth were performed to collect enough individuals. On each sampling date, 80–100 asexual females (when present) with a minimum body size of 1 mm (from the top of the eye to the base of the spine) were randomly selected, and the presence and number of eggs was documented. POC concentrations and water temperatures presented are modified from previous studies (Rellstab *et al.*, 2007; Rellstab and Spaak, 2007).

Starvation experiment

We performed a starvation experiment at 5°C, using a full factorial design with two-food treatments and two taxa from each of the three lakes described above. Food treatments were: no food (complete absence of food) and low food (adding $0.1\ mg\ C\ L^{-1}$ of chemostat-grown *Scenedesmus obliquus* two times a week). The POC level of the added food was determined by measuring its absorption with a photometer. We chose clones from two taxa from each lake mentioned above: parental *D. hyalina* (P), and hybrids of *D. hyalina*×*galeata* or their backcrosses, hereafter referred to as hybrids (H). Allozyme electrophoresis was used for taxonomic classification after Keller and Spaak (Keller and Spaak, 2004). Individuals were assayed for the enzyme loci AO and AAT, which are diagnostic markers to distinguish between *D. galeata* and *D. hyalina*, and the PGI and PGM loci, which can be further used to identify multi-locus genotypes.

We randomly chose three clones per taxon in each lake, with different multi-locus genotypes (or a different sampling year, if this was not possible, see Table I). An exception was parental *D. hyalina* of Greifensee, where we had only two clones available. Five replicates were used

Table I: Overview of the clones used in the starvation experiment and their body size on the starting day of the experiment

Clone	Lake	Taxon	AO	AAT	PGI	PGM	Sampling year	Size day 1 (mm ± SE)
BRZ H1	Brienz	Backcross *to D. hyalina*	ss	sf	mm	ff	2003	0.62 ± 0.01
BRZ H2	Brienz	Backcross *to D. hyalina*	ss	sf	ss	ff	2003	0.63 ± 0.02
BRZ H3	Brienz	*D. hyalina*×*galeata* F1	sf	sf	mm	ff	2004	0.59 ± 0.01
BRZ P1	Brienz	Parental *D. hyalina*	ss	ss	mm	ff	2003	0.69 ± 0.01
BRZ P2	Brienz	Parental *D. hyalina*	ss	ss	mm	f+f+	2005	0.66 ± 0.01
BRZ P3	Brienz	Parental *D. hyalina*	ss	ss	sm	ff+	2005	0.66 ± 0.01
CON H1	Constance	*D. hyalina*×*galeata* F1	sf	sf	mm	sf	2003	0.69 ± 0.01
CON H2	Constance	*D. hyalina*×*galeata* F1	sf+	sf	mm	ff	2003	0.66 ± 0.01
CON H3	Constance	*D. hyalina*×*galeata* F1	sf+	sf	mm	mm	2001	0.67 ± 0.00
CON P1	Constance	Parental *D. hyalina*	ss	ss	mm	ff	2001	0.66 ± 0.01
CON P2	Constance	Parental *D. hyalina*	ss	ss	mm	ff+	2001	0.69 ± 0.01
CON P3	Constance	Parental *D. hyalina*	ss	ss	mm	ff	1998	0.70 ± 0.01
GRE H1	Greifensee	*D. hyalina*×*galeata* F1	sf	sf	mm	sm	2002	0.54 ± 0.00
GRE H2	Greifensee	*D. hyalina*×*galeata* F1	sf	sf	mf	mf	2002	0.64 ± 0.01
GRE H3	Greifensee	Backcross *to D. hyalina*	ss	sf	mm	ff	2003	0.62 ± 0.01
GRE P1	Greifensee	Parental *D. hyalina*	ss	ss	mm	ff	2003	0.63 ± 0.01
GRE P2	Greifensee	Parental *D. hyalina*	ss	ss	mm	sf	2003	0.62 ± 0.00

Taxa were determined by allozyme electrophoresis using the two species specific markers AO and AAT. Alleles in AO, AAT, PGI and PGM: s = slow, m = medium, f = fast, f+ = very fast.

for each clone. This resulted in 170 experimental units. Each unit consisted of a single neonate that was placed in a 100 mL jar. Mortality and fecundity (number of juveniles born) for all animals were recorded at least three times per week. If juveniles were born, they were separated from their mother and put into a no food treatment.

Only second and third clutch juveniles born within 24 h of adult second and third clutch females (originating from one single mother per clone) were used. These mothers were reared at 12°C in filtered (0.45 μm) lake water, fed by adding 1.0 mg C L^{-1} three times a week. Because the age at first reproduction fluctuated broadly between clones, lakes and taxa (16–24 days), the starting day of the experiment differed among clones. For each clone, initial body size was obtained from 10 juveniles that were not used in the experiment.

To simulate winter conditions, we performed the experiment (including water exchange) in a walk-in growth chamber set at 5°C with an 8:16 hour light:dark photoperiod. We used filtered (0.45 μm) water taken from oligotrophic Lake Lucerne in December 2004, in order to have as little dissolved phosphorus as possible and so that none of the clones would have the advantage of experiencing water from its lake of origin during the experiment. Water was changed once a week. The experiment lasted from 28 March to 3 August 2005.

Statistical analysis

To evaluate differences in the body size of the neonates on day 1, we performed a three-way ANOVA with lake and taxon as fixed factors, and clone (nested within the lake and taxon) as random factor. Pearson's *r* was calculated separately for both food treatments to test whether the average body size of the neonates correlated with the average lifespan and population growth rate *r* for each clone. We used a four-way ANOVA to test the effect of clone (random factor, nested within lake and taxon), taxon, lake and food concentration (all fixed factors) on lifespan and on *r*. Analyses of lifespan were based on individuals, whereas analyses of *r* were done on a clonal level.

The population growth rate *r* (intrinsic rate of increase) was calculated for each clone that reproduced using the Euler–Lotka equation:

$$1 = \sum_{x=1}^{n} l_x m_x e^{-rx}$$

where l_x represents the age-specific survivorship of the clone, m_x is the average number of offspring per surviving individual and x is the age (in days). In clones where no offspring were produced, we calculated an average population growth rate *r* by using the following formula: $r = (\ln(N_x)/\ln(N_0))/x$ where $N_x \equiv 0.01$ (number of animals at the end of the experiment), $N_0 \equiv 5.01$ (number of animals at the start of the experiment) and x the number of days until all animals of the clone had died. The constant value of 0.01 was added to N to make the calculation feasible.

To test whether there was a correlation between the lifespan of the mother and its offspring, Pearson's *r* was calculated. All error values given in the text represent the standard error.

RESULTS

Field data of Lake Brienz

Lake Brienz is a cold and unproductive system, especially in winter. From 2003 to 2006, average water temperature (Fig. 1A) in the upper 30 m never exceeded 12°C (epilimnetic summer temperature reached 15–

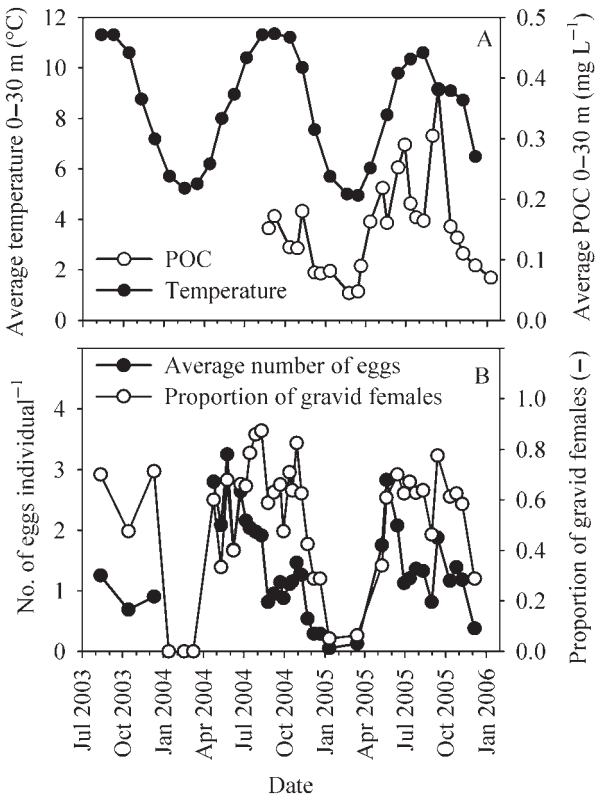

Fig. 1. (**A**) Average water temperature (black) and average POC concentration (white) of Lake Brienz from August 2003 to January 2006 (upper 30 m). Data modified from Rellstab *et al.* (2007) and Rellstab and Spaak (2007). (**B**) Proportion of gravid females (white) and average number of eggs of these females (black) in Lake Brienz from August 2003 to January 2006.

20°C in summer), and dropped below 5–6°C from January to March. Average POC concentrations (Fig. 1A) rarely reached 0.2 mg C L^{-1} in summer and were below 0.1 mg C L^{-1} for 5 months in winter (complete dataset only for 2004). These data served as a basis for the POC treatments and water temperature used in the experiment. During winters of the study period, *Daphnia* density dropped below the detection level of the routine sampling program for several consecutive months, reaching population growth rates of -0.10 to -0.15 $days^{-1}$ (Rellstab *et al.*, 2007). However, individuals were always present in the qualitative samples used for this study, as a larger volume of filtered water was analysed. Fecundity data of adult females of *Daphnia* (Fig. 1B) follow the dynamics of temperature and carbon concentration. Fecundity sharply decreases with the start of winter. In winter 2004, no gravid females could be found for 3 months; in winter 2005, gravid females were rarely found. Fecundity increased again in April in both years.

Starvation experiment: body size of neonates

The body size of neonates differed significantly ($P < 0.05$) between lakes, taxa and clones (nested within lake and taxon). Lake Constance neonates were the largest, followed by Lake Brienz and Greifensee (Table I). Parental *D. hyalina* neonates were larger than hybrids. The correlation between body size of neonates and lifespan of *Daphnia* was close to significance in the no food treatment ($n = 17$, Pearson's $r = 0.451$, $P = 0.069$), but not in the low food treatment ($n = 17$, Pearson's $r = 0.252$, $P = 0.329$). Moreover, body size of neonates was significantly correlated to population growth rate r in the no food treatment ($n = 17$, Pearson's $r = 0.483$, $P < 0.05$), but not in the low food treatment ($n = 17$, Pearson's $r = 0.226$, $P = 0.383$).

Starvation experiment: lifespan

In both food treatments, the first juveniles died after 4 days. Greatest lifespan was 36 days (individual from CON P3) without food and 126 days (individual from CON H3) under low food conditions. Considering survival of the experimental animals over time, irrespective of the taxon and clone, Lake Brienz and Greifensee *Daphnia* showed high mortality in the no food treatment starting on day 5; all animals finally died within 22 or 13 days, respectively (Fig. 2). Lake Constance clones performed better under no food conditions; the first animal died on day 10. Mortality then increased strongly, but slowed down after day 14. In the low food treatment, a consistent pattern can be seen for all lakes: high mortality on days 9–11, followed by low mortality, again a phase of high mortality from days 17–20, again followed by low mortality. Thereafter, Lake Constance and Greifensee animals did not show high mortality until around the period when some animals produced their first clutch (days 66 and 90, respectively).

The results of the four-way ANOVA are presented in Table II and the average lifespan of the different clones are shown in Fig. 3. Food concentration had a highly significant influence on the lifespan of *Daphnia*: individuals without food had much shorter lifespans (12.2 ± 0.8 days) compared to those in the low food treatment (43.3 ± 4.1 days). Lake Constance animals lived the longest (37.6 ± 4.9 days), followed by Greifensee (26.1 ± 4.2 days) and Lake Brienz (19.3 ± 2.6 days). However, lake origin was not a significant factor in the statistical analysis. In the no food treatment (Lake Constance 17.2 ± 1.7 days > Lake Brienz 10.8 ± 0.9 days > Greifensee 7.9 ± 0.4 days) was the order of survival success different than in the low food treatment (Lake Constance 58.0 ± 8.2 days > Greifensee 44.4 ± 6.6 days > Lake Brienz 27.9 ± 4.6 days). Lake Brienz clones clearly showed the smallest reduction in lifespan between the low and no food treatment, compared to the clones of the other two lakes. There was also a significant interaction effect of clone and lake origin. The effect of the taxon and all other interactions were not significant in either analysis.

Starvation experiment: reproductive success and population growth rate *r*

None of the animals in the no food treatment produced offspring, whereas in the low food treatment a total of 25 neonates were born. Brood size ranged from 1 to 5 (individual from clone GRE P2) eggs. No ephippia were produced in any treatment. Population growth rate r could only be calculated using the Euler–Lotka equation in the clones that reproduced (BRZ P2, CON H1, CON H3, GRE H2, GRE P2). No animals were able to produce a second clutch before they died. As a result of this fact and the low clutch size, r was only slightly positive in clones CON H3 and GRE P2. All other clones showed a negative population growth rate in both food treatments (Fig. 4). All clones except BRZ H3 showed higher population growth rates in the low food treatment (average -0.05 ± 0.02 $days^{-1}$) compared to conditions without food (-0.22 ± 0.02 $days^{-1}$). The four-way ANOVA revealed a highly significant effect of the food treatment and of clone on the population growth rate r of *Daphnia* (Table II). Moreover, the interaction of Lake × Food was significant.

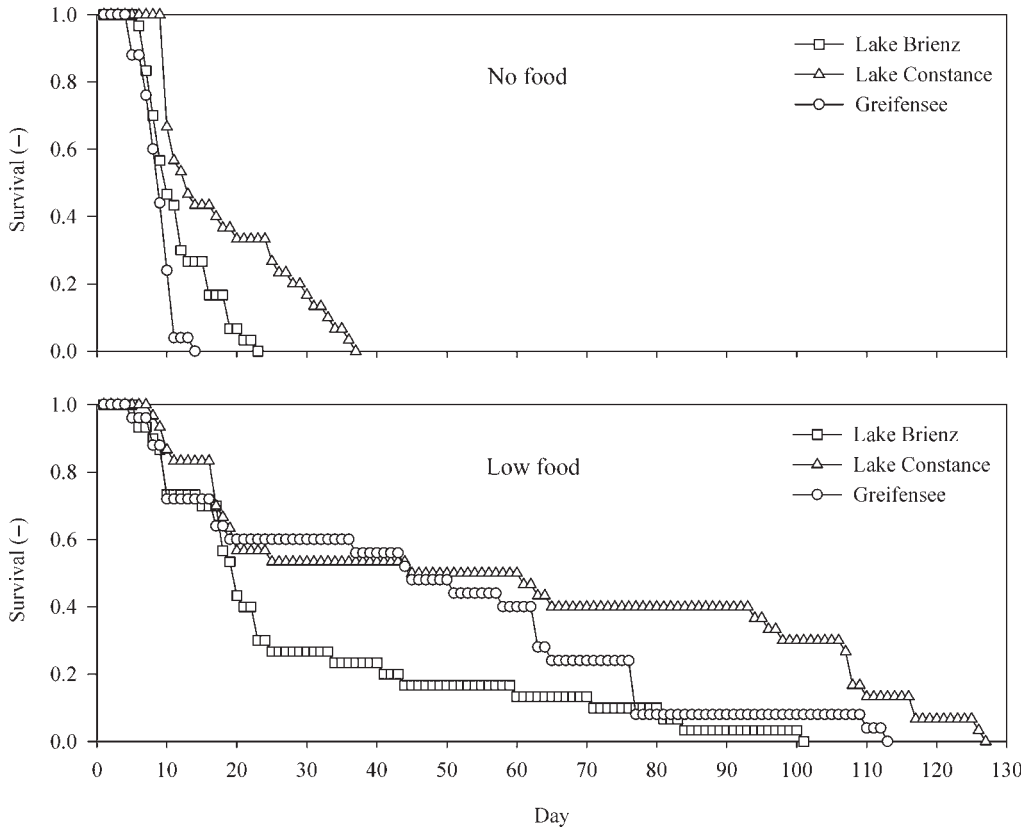

Fig. 2. Survival curves of *Daphnia* originating from three different lakes under no food (top) and low food (bottom) conditions.

Table II: Results of the four-way ANOVA testing the effect of clone, lake, taxon and food treatment on the lifespan (left) and population growth rate r (right) of Daphnia

Dependent variable	Lifespan				Population growth rate r			
Effect	df	MS	*F*	*P*	df	MS	*F*	*P*
Intercept	1	129284.9	57.0	*<0.001*	1	0.601	74.537	*<0.001*
Clone (Lake × Taxon)	11	2266.2	1.78	0.192	11	0.008	3.383	*0.027*
Lake	2	5063.3	2.2	0.153	2	0.012	1.440	0.278
Taxon	1	1030.9	0.5	0.514	1	0.004	0.441	0.520
Food	1	41796.6	31.6	*<0.001*	1	0.217	91.225	*<0.001*
Clone (Lake × Taxon) × Food	11	1320.3	2.8	*0.002*	11	0.002		
Lake × Taxon	2	708.7	0.3	0.738	2	0.001	0.069	0.934
Lake × Food	2	2392.5	1.8	0.209	2	0.025	10.887	*0.002*
Taxon × Food	1	872.7	0.7	0.433	1	0.001	0.327	0.578
Lake × Taxon × Food	2	301.5	0.2	0.800	2	0.001	0.223	0.803
Error	136	468.4			0			

Significant effects are highlighted in italic.

Whereas Greifensee clones had a higher r in the low food treatment (-0.02 ± 0.03 days^{-1}) than Lake Constance and Lake Brienz clones (-0.05 ± 0.03 days^{-1} and -0.09 ± 0.03 days^{-1}, respectively), they had a lower r in the no food treatment (-0.30 ± 0.03 days^{-1}) compared to Lake Constance and Lake Brienz clones (-0.15 ± 0.03 days^{-1} and -0.20 ± 0.03 days^{-1}, respectively). All other factors and their interactions were not significant.

Age at first reproduction differed greatly between lakes. It took an average of 66 days for the BRZ and GRE clones to produce neonates, while CON clones needed around 90 days.

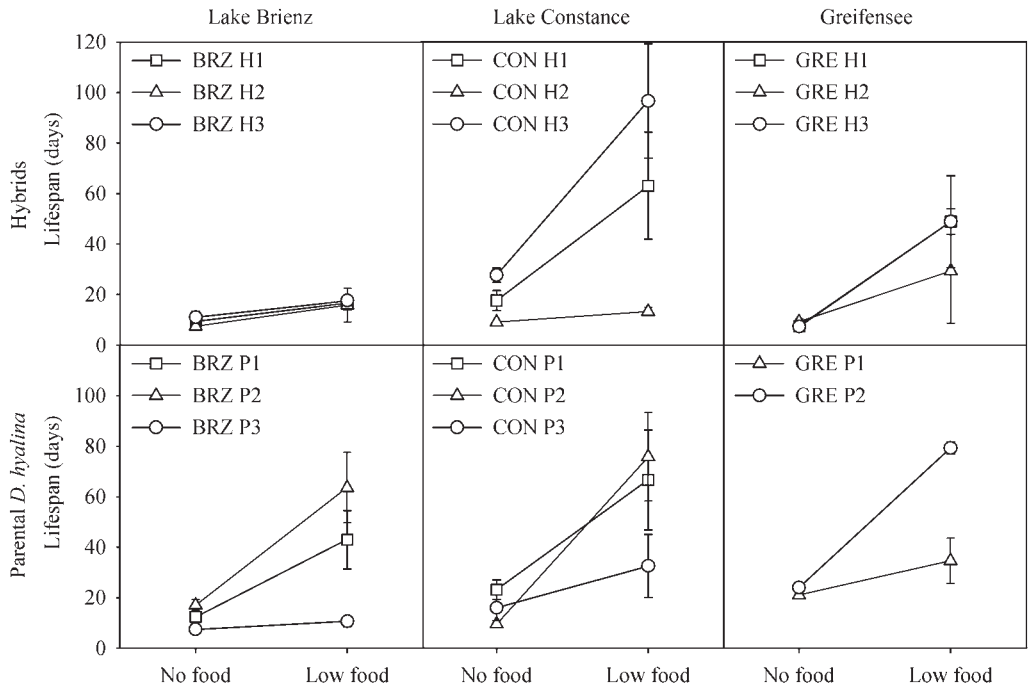

Fig. 3. Average lifespan of the different clones in the starvation experiment under no food and low food conditions. Clones belong to three different lakes (left to right) and two different taxa (top and bottom). Error bars represent standard error.

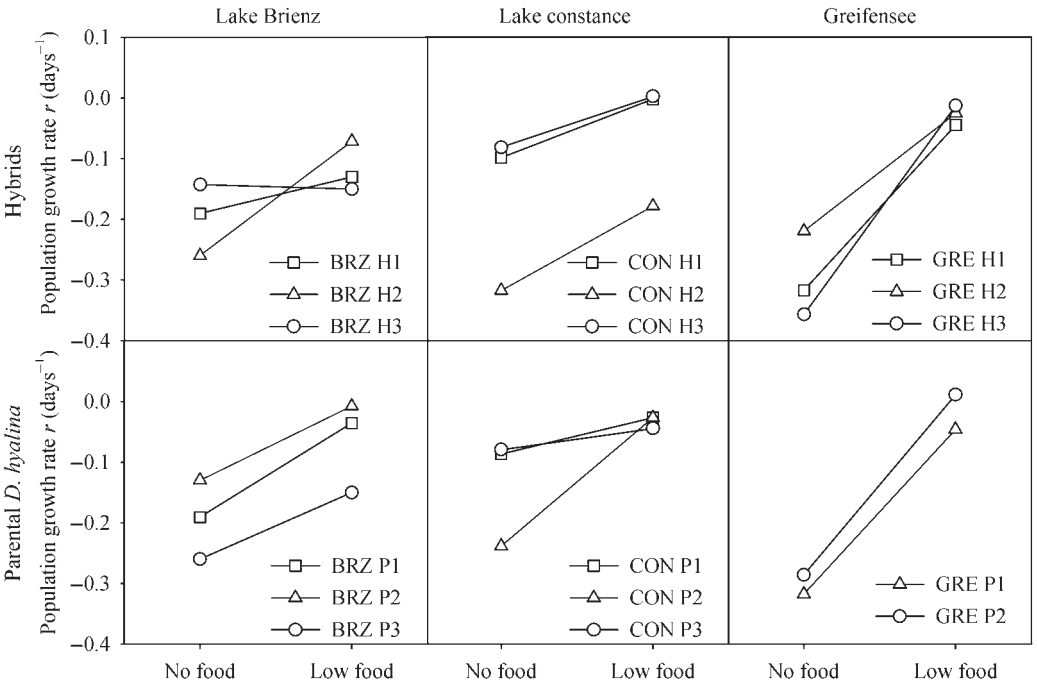

Fig. 4. Population growth rate *r* of the different clones in the starvation experiment under no food and low food conditions. Clones belong to three different lakes (left to right) and two different taxa (top and bottom).

Starvation experiment: offspring survival

Offspring produced during the experiment were put into a no food treatment. Lifespans of these juveniles ranged between 2 and 25 days (average lifespan per brood between 2.0 and 23.5 days). Juveniles from Lake Constance lived the longest (16.3 ± 2.3 days), followed

by Lake Brienz (6.0 ± 0.0 days) and Greifensee (4.9 ± 0.5 days). There was a significant and strong correlation ($n = 25$, Pearson's $r = 0.742$, $P < 0.001$) between the lifespan of the mother and the lifespan of her offspring.

DISCUSSION

Our results show that *Daphnia* can be resistant to starvation for long periods at low temperature, which indicates that they can successfully overwinter as parthenogenetic animals in ultra-oligotrophic lakes. The taxon and lake from which the daphnids originate did not significantly influence the survival under winter conditions, but regarding population growth rate, the interaction between lake origin and food concentration was significant. Reproduction took place only under low food conditions, but was in the majority of cases not high enough to result in a positive population growth rate. The lifespan of the neonates that hatched during the experiment was significantly correlated to the lifespan of their mothers.

Despite significant differences in size at birth at the start of the experiment, we found no significant correlation between the body size and the lifespan. However, larger neonates had a significantly higher population growth rate *r* in the no food treatment. Body size can affect the resistance of neonates to starvation (Tessier and Consolatti, 1989), following the predictions of the size-efficiency hypothesis (Brooks and Dodson, 1965). Another possible reason is that larger neonates contain more energy reserves, as discussed in Tessier and Consolatti (Tessier and Consolatti, 1989). In contrast, Glazier (Glazier, 1992) found no correlation of the body size of neonates and the ability to survive starvation in *D. magna*.

Daphnia in our experiment showed an average lifespan of 12 days (maximum 36 days) under no food conditions and 43 days (maximum 126 days) under low food conditions (Figs 2 and 3). This is higher than in other starvation studies, where a lifespan under complete absence of food was on average 6–9 days for juvenile daphnids (Gliwicz, 1991; Gliwicz and Guisande, 1992; Perrin *et al.*, 1992) and 5–11 days for adult daphnids (Lemcke and Lampert, 1975; Threlkeld, 1976; Elendt, 1989) of various *Daphnia* species. Only Epp (Epp, 1996) reported lifespans of 28 days and more, using neonates of *D. pulicaria* in the complete absence of food. All of these other experiments were performed at 15–20°C, so the low water temperature in our experiment (5°C) is most likely responsible for the differences in lifespans. As we used filtered lake water (as most of the studies mentioned in this manuscript), it is also possible that the daphnids in the no food treatment had very low amounts of organic matter other than algae available, e.g. bacteria, which are known to be a potential food source (Kankaala, 1988). The lifespans at low food treatments, which represent realistic conditions for an ultra-oligotrophic lake in winter, show that it is possible for at least some *Daphnia* clones to overwinter successfully as parthenogenetic animals.

The food concentration had a significant effect on the lifespan of daphnids, whereas lake and taxon did not play a substantial role. We also found a significant interaction of clone origin and food concentration, meaning that clones showed different patterns in response to different food concentrations. Inter-clonal variation in resistance to starvation was also shown by Epp (Epp, 1996). Surprisingly, it was not the clones from ultra-oligotrophic Lake Brienz, but the clones from mesotrophic Lake Constance that had the highest lifespans under no food conditions. Lake Brienz clones performed slightly better than clones from eutrophic Greifensee. Maternal effects, which can be environmental or genetic (Räsänen and Kruuk, 2007), are most likely the cause of the variation in lifespan. These play an important role in the resistance to starvation because mothers transfer energy reserves like lipids to their offspring (Tessier *et al.*, 1983; Cowgill *et al.*, 1984), and produce neonates of different sizes (Gliwicz and Guisande, 1992). Especially in our no food treatment, neonates depended only on the energy reserves received from their mothers. Prior to our experiment intra- and inter-clonal differences due to maternal effects resulting from different environmental conditions were eliminated, because several generations were treated exactly the same before their juveniles were exposed to the treatments. Still, as the mothers and grandmothers of our experimental animals were reared at the same relatively high food concentration, our results could partially represent the efficiency at which the mothers feed at high food concentration and transfer lipids to their juveniles. The strong correlation between the survival of *Daphnia* that reproduced in the low food treatment and the survival of its offspring in the no food treatment, and the significant effect of neonate body size on population growth rate, further support the importance of (genetic) maternal effects.

By calculating the population growth rate *r* for each clone in both food treatments, we obtained a measure based not only on survival, but also on reproduction. Only two clones managed to produce enough offspring to have a positive population growth rate, all others were negative. With daily death rates of up to 36% under no food and up to 18% under low food, our results are similar to or slightly lower than death rates found in other

studies (Lemcke and Lampert, 1975; Lynch, 1989; Rohrlack *et al.*, 1999). Our ANOVA showed a significant effect of food treatment and its interaction with lake origin on population growth rate *r*. Moreover, there was a significant inter-clonal variation. There are remarkable differences in the outcome of the statistical analyses concerning lifespan and population growth rate. Because our population growth rates are also mostly derived from lifespan (we could calculate *r* using the Euler–Lotka equation in only five clones), these differences must come from the fact that the temporal distribution of deaths is not incorporated. It represents the average growth rate from the start of the experiment until the last individual of the clone had died.

Assuming that the variation in the lifespan and growth rate is determined mainly by genetic maternal effects, we could use the difference of the response (reaction norm) to the two food treatments as a measure of resistance to starvation. Our ANOVAs showed a significant Lake × Food interaction for *r*, meaning that the reduction in population growth rate is different among lakes when comparing the low and no food treatment. If we look at lifespan, *Daphnia* clones from Lake Brienz show a remarkably smaller difference between the two food treatments (16.4 days) compared to those from Greifensee (36.5 days) or Lake Constance (40.8 days). The reduction of population growth rate *r* is much higher in Greifensee clones (0.28 $days^{-1}$) than in clones from Lake Constance (0.10 $days^{-1}$) or Lake Brienz (0.10 $days^{-1}$). This is an indication that, when looking at lifespan, *Daphnia* from Lake Brienz perform best, and when considering population growth rate, *Daphnia* from Greifensee are performing worst. In fact, a three-way ANOVA with Clone (random factor, nested in Lake × Taxon), and Lake and Taxon as fixed factors shows a significant lake effect ($df = 2$, $F = 10.886$, $P < 0.05$) on the difference in population growth rate (but not lifespan) between the two food treatments. This agrees with our original prediction that clones from an ultra-oligotrophic lake show higher fitness under starvation.

Taxa of the *D. hyalina/galeata* species complex differ in many life history traits, including migration behaviour (Weider and Stich, 1992), timing and extent of sexual reproduction (Jankowski and Straile, 2004), genetic diversity (Jankowski and Straile, 2004; Keller and Spaak, 2004), parasite prevalence (Wolinska *et al.*, 2006), predation sensitivity (Spaak *et al.*, 2000) and habitat preference (Keller *et al.*, 2008). We could not verify our hypothesis that parental *D. hyalina*, which are the dominant taxon in oligotrophic lakes (Keller *et al.*, 2008), would perform better under starvation conditions than hybrids. Why could we not find an effect? Several reasons might play a role. First, the taxonomic composition present in these lakes might not be determined by the ability of a taxon to resist starvation in winter. Other selection factors, such as feeding efficiency under better food conditions or overwintering success in diapause could be more important. Secondly, the clones used in this experiment were not collected specifically at the end of winter when we would expect the better adapted clones to be more abundant. Thirdly, using two species specific markers can lead to misidentification of taxa due to backcrossing of hybrids with the parental taxa. A part of parental *D. hyalina* or *D. hyalina* × *galeata* clones could actually represent backcrosses to *D. hyalina* as is most likely the case in Greifensee for *D. hyalina* (Keller and Spaak, 2004) and in Lake Brienz for *D. hyalina* × *galeata* (C. Rellstab, unpublished results).

Field data from Lake Brienz presented here and in a previous study (Rellstab *et al.*, 2007) show that winter is the major bottleneck for a *Daphnia* population in an ultra-oligotrophic lake. Temperature and food concentration are at very low levels, resulting in low fecundity and negative population growth rates (similar to the ones found in our experiment) for several consecutive months. However, asexual females are always present throughout the winter and represent an important part of the starting population in spring, when conditions ameliorate. Due to the low population density (reducing the chances of mating), the large depth and the high sedimentation rate (reducing hatching success), it is unlikely that overwintering by sexually produced resting eggs is a successful strategy. Note that under natural conditions, the effect of predation by fish on the asexually overwintering population should not be underestimated (Jeppesen *et al.*, 2004), although in Lake Brienz predation pressure is low (Müller *et al.*, 2007b). Even low predation pressure could have a strong impact due to the low population growth rates found in our experiment.

Since the growth rates in our experiment are mostly negative, it appears that it is the individual ability to withstand starvation in winter, rather than significant population growth, that permits clones to be present in spring. Thus, the probability of a female's offspring surviving the period of starvation must, at least in some years, exceed their probability of surviving through winter as diapausing eggs and then hatching successfully. As the trophic state of the lake changes, previous high-fitness life history strategies might become poorly adaptive. The clonal variation we showed may provide the basis for evolutionary response by *Daphnia*, but will depend upon the strength of selection and the available genetic variation (Kinnison and Hairston, 2007). In Lake Brienz, oligotrophication has led to substantial declines in fish stocks (Müller *et al.*, 2007a, 2007b). Understanding how their major food resource survives and adapts to the stressful conditions of generally

decreasing food conditions will be critical in evaluating how to manage the balance between improved water quality and the needs of the fishery.

In conclusion, we have shown that the response of *Daphnia* to realistic winter conditions is highly variable among clones, and is influenced mainly by food concentration and by the lake origin. Taxon did not play a significant role. The absolute performance of the different clones did not match our expectation that clones from unproductive systems would perform better under their local conditions. Still, the significant interaction of lake origin and food concentration indicates that these clones perform better if we look at the difference of the response to the two food conditions. Moreover, considering results found in the literature and from our experiment, there is evidence that maternal effects play a major role, especially when neonates are exposed to the complete absence of food. Our results show that it is possible for *Daphnia* clones to withstand long periods of low food conditions (typical for ultra-oligotrophic systems in winter), and short periods of complete starvation. Reproduction increases population growth rate until conditions ameliorate, but does not necessarily result in positive growth, since clutch sizes are too small and egg development time is too long.

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